Protocol of RNA extraction

Kit content:

RNeasy Mini Spin Columns

Collection Tubes (2 ml)

Buffer RLT Buffer RW1 Buffer RPE

RNase-Free Water

Process:

1. Harvest the cells appropriate number of cells (maximum 1 x 10^9)by centrifuging at 5000 x g for 3-5 min at 4°C. Discard supernatant ensuring all media is completely removed.

2. Resuspend cells in 100 µl lysozyme-containing TE buffer by vortexing.

	Lysozyme concentration in TE buffer	Incubation time at room temperature
Gram-negative bacteria	400µg/ ml	3-5 min
Gram-positive bacteria	3mg/ ml	5-10 min

3. Add 350 µl Buffer RLT and vortex vigorously to lyse the spheroplasts. If insoluble material is visible, centrifuge for 2 min at full speed, and use only the supernatant in subsequent steps.

4. Add 250 µl ethanol (96–100%) to the homogenized lysate, and mix well by pipetting without centrifugation.

5. Apply sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm).

6. Add 700 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash

7. Transfer RNeasy column into a new 2-ml collection tube. Pipet 500 µl Buffer RPE onto the RNeasy column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash

8. Centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.

9. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥ 8000 x *g* (≥10,000 rpm) to elute the RNA.